meninges and blood vessels. After homogenization of the tissue in acetone, lipids were removed by treatment with chloroform-methanol (2:1, w/v). The residue was dispersed in 0.2M-tris-HCl buffer, pH 8.0, containing Ca^{2+} ions, and incubated at 60° for 24 hr. in the presence of Pronase. Polyanions were precipitated from the digest by the addition of cetylpyridinium chloride. After careful removal of nucleic acid, the glycosaminoglycans were again precipitated by the addition of cetylpyridinium chloride and separated into three fractions that differed in their solubility in aqueous potassium chloride (Scott, 1963).

The nature and relative amounts of the glycosaminoglycans present in each of the three fractions were determined by chemical analysis, electrophoresis on cellulose acetate (Stefanovich & Gore, 1967) and chromatography on cellulose columns (Svejar & Robertson, 1967). The hydrolysis of the susceptible glycosaminoglycans by testicular hyaluronidase was investigated, the extent of hydrolysis being estimated by chemical analysis, separation of the products by electrophoresis on cellulose acetate and chromatography on cellulose columns.

The results indicate that the following glycosaminoglycans are present in all the animal brain tissues examined; hyaluronic acid, heparan sulphate, chondroitin sulphate and dermatan sulphate.

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Phosphoprotein-bound Iron in the Plasma of the Laying Hen

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Plasma iron in the laying hen and the oestrogenized non-laying bird is 200–500% higher than in the non-laying bird (Ramsay & Campbell, 1954, 1956). About half the total appears to be transferrin-bound. Rodriguez & Planas (1964) suggested that the remainder is bound to conalbumin, the iron-binding protein characteristically found in egg-white, but their evidence overlooks the similarity in properties between conalbumin and transferrin. The phosphoprotein phosvitin has iron-binding properties (Taborsky, 1963), and Greengard, Mendelsohn & Gordon (1965) suggested without direct evidence that the non-transferrin iron in the

plasma of oestrogenized cockerels might be bound to phosphoprotein.

Magnesium carbonate fails to remove transferrin-bound iron from plasma (Ramsay, 1957), but it has now been found to remove all the phosphoprotein phosphorus and about half the total iron from the plasma of laying hens and oestrogenized cockerels. Gel filtration on Sephadex G-200 in 0.15M NaCl–5mM-tris-HCl buffer, pH 7.4, and ion-exchange fractionation on DEAE-cellulose in 5mM-tris-HCl buffer, pH 8.0, with an NaCl gradient (0.1–0.5M) both separate the iron into two fractions, of which one migrates with transferrin and the other with phosphoprotein. Combinations of these techniques lend further support to the view that the non-transferrin iron is associated with phosphoprotein and not with conalbumin.

When ^{59}Fe -labelled plasma from a normal cockerel is mixed with plasma from a laying hen, the non-transferrin iron in the latter does not become labelled in 4 hr. at 37°, nor is there any fall in the specific radioactivity of the transferrin iron. There is thus no exchange between the fractions *in vitro*. On the other hand, specific radioactivity measurements made at intervals for several hours after the intravenous injection of [^{59}Fe]-transferrin (normal cockerel plasma labelled *in vitro*) show that in the laying hen and the oestrogenized cockerel transferrin iron serves as a precursor of phosphoprotein iron (Zilversmit, Entenman & Fishler, 1943), presumably by some process other than simple transfer in the plasma.

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Disulphide Bonds of Ovalbumins

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Hen and duck ovalbumins differ in disulphide content (Gilmore & Fothergill, 1967) and show large differences in 'fingerprint' pattern (Fothergill & Perrie, 1966), although their immunological cross-reactivity suggests marked structural similarity. The present study was undertaken to examine the location of the disulphide bond of hen ovalbumin

and to compare this with other avian ovalbumins. Cysteine-containing regions of the ovalbumin polypeptide chain were located by labelling with iodo[^{14}C]acetate after mercaptoethanol reduction, followed by tryptic digestion and high-voltage electrophoresis. It was found that one of the hen ovalbumin cysteine peptides was the C-terminal peptide and that the cysteine residue was the fourth amino acid from the C-terminus (Fothergill & Gilmore, 1968). All the labelled peptides from duck ovalbumin contained lysine or arginine. The involvement of this C-terminal peptide in the disulphide bond of hen ovalbumin was investigated by using a double-labelling technique.

Hen ovalbumin was carboxymethylated with iodo[^{14}C]acetate in 8M-urea (Crestfield, Moore & Stein, 1963) but without the addition of reducing agent. After removal of the unchanged iodo[^{14}C]acetate by gel filtration, the carboxymethylated ovalbumin was treated with mercaptoethanol and iodo[^3H]acetate. The doubly labelled protein, containing approximately 4g.atoms of ^{14}C and 2g.atoms of ^3H /mole, was heat-denatured and digested with trypsin. The tryptic peptides were separated by high-voltage electrophoresis at pH 6.5 on Whatman no. 3 MM paper. The electrophoresis paper was cut transversely at 1cm. intervals and ^{14}C and ^3H were measured by using a liquid-scintillator double-counting technique. ^3H was found in the C-terminal peptide and in the pH 6.5 neutral region, whereas only a small amount of ^{14}C was located in the C-terminal peptide.

We conclude that the cysteine residue close to the carboxyl terminus of hen ovalbumin is involved in the disulphide bond, and that duck ovalbumin probably lacks this cysteine residue. We are investigating by similar techniques other ovalbumins from birds that are more closely related phylogenetically to the hen.

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Binding of Glutathione and Cysteine to Cellular Protein by Mixed Disulphide Bonds

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It has been demonstrated by Modig (1968) that protein-bound GSH and cysteine are present in

Ehrlich ascites cells in amounts that form about one-third of the total cellular content of these thiols. The present study investigated the distribution of mixed disulphides in normal tissues, and their mechanism of formation. We attempted also to assess their involvement in the turnover of cellular free thiol.

High-speed-supernatant fractions were obtained from rat spleen, heart, liver, skeletal muscle, kidney and Yoshida ascites sarcoma. The preparations were dialysed, to remove free thiol and disulphide, and analysed for mixed disulphide by a modification of the method of Modig (1968). Extracts were reduced with sodium borohydride in borate buffer, pH 9.0, containing 8M-urea. After deproteinization with sulphosalicylic acid, GSH was estimated by the method of Ball (1966), and total thiol by the method of Ellman, as modified by Harrap (1967). Mixed disulphides were found in all the tissues examined. Concentrations of the protein-GSH mixed disulphide in the different tissues were similar, but amounts of the protein-cysteine disulphide were more variable.

To examine the ability of tissues to reduce mixed disulphide, substrates were prepared from bovine plasma albumin with GSH or cysteine. Homogenates of Yoshida ascites cells, and of kidney, spleen and heart were found to reduce the albumin-GSH disulphide in the presence of NADPH and to a much lesser extent with NADH. During this enzymic reaction, GSH and protein thiol groups were released in equimolar ratio. Little or no such activity was found in erythrocytes, skeletal muscle or liver. None of the tissues examined would reduce the albumin-cysteine disulphide under these conditions.

An attempt was made to test the speculation that these compounds might act as intracellular reserves of thiol compounds by using Yoshida ascites sarcoma cells in which a sixfold increase of cellular GSH content had been induced by chlorambucil treatment (Harrap & Hill, 1968). Under these conditions, far from decreasing as the free thiol concentration increased, both protein-GSH and protein-cysteine mixed disulphides doubled in concentration.

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